PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GCN/PG5043	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/15004	International filing date *(day/month/year)* 10.12.2003	Priority date *(day/month/year)* 13.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant GLAXO GROUP LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.06.2004	14.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Schmitt, C Telephone No. +49 89 2399-7351

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/15004

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21 as originally filed

Sequence listings part of the description, Pages

1-2 as originally filed

Claims, Numbers

1-18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-11, 13-17

   because:

   ☒ the said international application, or the said claims Nos. 1-9 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 9-11, 13-17 (all partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	9-11, 13-16 (all partially), 12, 18
Inventive step (IS)	Yes:	Claims	
	No:	Claims	9-11, 13-16 (all partially), 1-8, 12, 18
Industrial applicability (IA)	Yes:	Claims	10-16 (all partially), 18
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the opinion

The basis of for this opinion is the application as originally filed.

The present application relates to polymorphisms in the 5-hydroxytryptamine transporter (5-HTT) gene and phenotypes that are associated or correlated therewith and to the use of such polymorphisms to determine the susceptibility of an individual to gastrointestinal diseases, in particular Irritable Bowel Syndrome (IBS).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-8 relate to a method of determining the susceptibility to gastrointestinal diseases in an individual. In view of the description (page 11, lines 10-12), said method can be carried out in vivo. Claims 1-8 amount thus a diagnostic method practised on the human/animal body. Claims 1-8 relate therefore to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT.

Claim 9 relate to a method for treating a patient comprising administering a therapeutically effective amount of a 5HT ligand. Claim 9 relates therefore to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT.

Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of claims 1-9 (Article 34(4)(a)(i) PCT).

Furthermore, claims 9-11 and 13-17 were only partially searched due to a lack of clarity in the sense of Article 6 PCT (see Box I.2. of the ISR). An opinion on novelty, inventive step and industrial applicability of said claims will only be given with respect to the subject-matter that was searched.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or

industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO01/61039

D2: Pata et al, "Serotonin transporter gene polymorphism in Irritable Bowel Syndrome.", The American Journal of Gastroenterology, July 2002, 97, pp. 1780-1784.

D3: WO02/057306, published 25th July 2002

D4: Tate C. G., "Baculovirus-mediated expression neurotransmitter transporters." Methods in Enzymology, 1998, 296, pp. 443-455.

D5: Lesh et al., "Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region." Science, 1996, 274, pp. 1527-1531.

V.1. Document D1 (the reference in parentheses applying to this document) discloses that 5HT ligand, such as alosetron, ondansetron, granisetron, tropisetron, dolasetron, mirtazapine, itasetron, pancopride, zatosetron, azasetron, cliansetron, YM-144, RS17017, tegaserod, prucalopride, norcisapride, 4-amino-5-chloro-2-methoxy-N-(1-substituted piperidin-4-yl) benzamide, buspirone, piboserod, renzapride, E3620 and LY315535 (page 10, line 27-page11, line 4) are used for the treatment of Irritable Bowel Syndrome (page 6, lines 14-20). Individuals with IBS can be treated by administering such 5HT ligand in a therapeutically effective amount (page 10, lines 14-17 and page 19, lines 19-22).

It should be noted that individuals having IBS are considered as belonging to individuals susceptible to such a disease. Moreover, the way in which a patient has been diagnosed as being susceptible to a disease is not a limiting feature of claims 9-12, these being second medical use claims which are only characterized by the "5HT ligand" and by the disease to be treated.

Claims 9-12 are therefore not new in the sense of Article 33(2) PCT.

V.2. Furthermore document D1 discloses an insertion/deletion polymorphism in the 5' untranslated region of the 5-HTT gene, wherein the deletion polymorphism has SEQ ID NO: 1 and the insertion polymorphism has SEQ ID NO: 2 (page 20). Said insertion/deletion polymorphism corresponds to the insertion/deletion polymorphism in the 5' untranslated region of the 5-HTT gene disclosed in the present application as being correlated to the susceptibility to gastrointestinal disease and the sequences of SEQ ID NO: 1 and SEQ ID NO: 2 disclosed in document D1 are identical to the sequences of SEQ ID NO: 1 and SEQ ID NO: 2 of the present application.
Document D1 discloses primers (page 20, underlined sequences of SEQ ID NO: 1 or 2) which are suitable to detect a polymorphism as defined in any one of claims 2 to 3.
Lastly, document D1 discloses a kit comprising PCR primers which bind to region flanking the polymorphism (i.e. the polymorphic deletion /insertion variation in the 5' untranslated region of the 5-HTT gene) (page 18, lines 17-22) or an antibody that specifically binds to a predetermined polymorphic region (page 18, line 30-page 19, line 2). Said kit being suitable for diagnosing susceptibility to gastrointestinal disease.

Claims 13-15 are therefore not new in the sense of Article 33(2) PCT.

V.3. Document D2 discloses a method of determining whether the polymorphisms in two regions (variable tandem repeats and the 5-HTTLPR polymorphism) in the 5-HTT gene are linked to Irritable Bowel Syndrome (page 1780, col. 1).
Document D2 also discloses two primers (page 1781, col.1, 3rd paragraph of the section "Genotyping") which are suitable for the dection of the polymorphic insertion/deletion site in the 5' non-translated region of the 5-HTT gene.
Lastly, an amplified DNA corresponding to the 5-HTTLPR deletion in the promoter region of the SERT gene (page 1781, col.1, section "molecular analysis") is considered as an isolated polynucleotide comprising a polymorphism that causes susceptibility to IBS.

Claims 13, 15 and 18 are therefore not new in the sense of Article 33(2) PCT over the disclosure of document D2.

V.4. Document D5 discloses a polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence (page 1527, col.3, 2nd paragraph). Said polymorphism is identical to the one disclosed in the present application.
Document D5, discloses also that constructs in which a luciferase reporter gene is fused to

the 5'-flanking promoter sequence containing the l or s form of the 5-HTTLPR, corresponding to SEQ ID NO: 2 and SEQ ID NO: 1, respectively, is obtained (page 1527, col. 3, 4th paragraph and Note 14, page 1530, col. 3).
Two primers, corresponding to the two primers underlined in SEQ ID NO: 1 and SEQ ID NO: 2 on page 20 of the present application, are also disclosed in document D5 (page 1530, col. 3, note 10.).
Claims 13, 15 and 16 are therefore not new in the sense of Article 33(2) PCT over the disclosure of document D5.

V.5. Claims 1-8 appear to be new on the sense of Article 33(2) PCT because their features are not disclosed in any available prior art.

Document D3 is regarded as being the closest prior art to the subject-matter of independent claim 1, and discloses (the references in parentheses applying to this document) a method of diagnosing irritable bowel syndrome comprising detecting the expression profile of $P2X_7$ of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue (claim 26).
Claim 1 differs in that the typing of the 5-HTT gene or protein is used to determine wether individual is susceptible to gastrointestinal diseases.
The problem to be solved by present claim 1 may be seen as the provision of an alternative method of determining the susceptibility to a gastrointestinal disease, in particular IBS.
The solution provided by claim 1 is the typing of the 5-HTT gene or protein. However, the mere typing of the HTT gene or protein does not solve the afordefined problem. In particular, the mere detection of a polymorphism in the HTT-gene does not enable the skilled person to determine if the individual is susceptible to a gastrointestinal disease as most of the polymorphisms occurring across the genome are not linked to any phenotype, in particular a disease.

The present description discloses various polymorphisms in the 5-HTT gene (page 3, line 19-22, Table 1 and page 5, lines 7-10) that could be linked to gastrointestinal diseases. However, a single insertion/deletion polymorphism in the 5' non-coding region of the 5-HTT gene defined by SEQ ID NO: 1 and SEQ ID NO: 2 has been shown to be linked to IBS (e.g. examples 1 and 2). The applicant has shown that a link exist between individual being homozygous for the deletion (i.e. having two alleles with SEQ ID NO:1) and IBS.

As claim 1 is not limited to the determination of the genotype at a polymorphic insertion/deletion allelic site in the 5' non-coding region of the 5-HTT gene, wherein a homozygous deletion genotype indicates that the individual is susceptible to IBS, said claim is considered to lack an inventive step in the sense of Article 33(3) PCT (see also "Further comment", below).

The same applies to claims 2-8, which are dependent on independent claim 1. Claims 2-8 therefore not inventive in the sense of Article 33(3) PCT.

V.6. No opinion on novelty and inventive step can be given for claim 17 as said claim does not fulfill the requirements of Articles 5 and 6 PCT (see item **V.7.4.**, below).

V.7. Further comment.

V.7.1. The applicant has shown that a single polymorphism in the 5-HTT gene, an insertion/deletion polymorphism in the 5' non-coding region of the 5-HTT gene defined by SEQ ID NO: 1 and SEQ ID NO: 2, is linked to a particular gastrointestinal disease which is IBS (e.g. examples 1 and 2).
As most of the mutations/polymorphisms occurring across the genome are not linked to any phenotype, in particular a disease, typing a mutation/polymorphism which is linked to a gastrointestinal disease, such as IBS, would represent trial and error experimentation as there is no teaching in the present application as to any additional polymorphism associated with a gastrointestinal disease. Apart from said insertion/deletion polymorphism in the 5' non-coding region of the 5-HTT gene that is linked to IBS, the skilled person would not be able without undue burden to identify or screen other polymorphism(s) in the 5-HTT gene which would be linked to a gastrointestinal disease in accordance with the broad scope of claims 1-8.
Even if it routine experimentation to identify other polymorphic site in the 5-HTT gene, the skilled person is however not able **without undue burden** to identify among the polymorphic sites thus identified which of these, if any, are linked to a gastrointestinal disease.
Thus, claims 1-8 are not supported under Article 6 PCT and the application does not meet the requirements of Article 5 PCT.

V.7.2. Furthermore, it is clear from the description on page 19 (example 2) that the correlation between the genotype of the individual and IBS is essential to the definition of the invention.

Since independent claim 1 does not contain these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

V.7.3. An isolated 5-HTT polynucleotide/protein only defined in terms of functional features (i.e. a polymorphism that causes gastrointestinal disease or a naturally occurring polymorphism that is in linkage disequilibrium therewith) is not sufficient to clearly and unambiguously characterize said polynucleotide or protein.
Claim 15 lacks therefore clarity in the meaning of Article 6 PCT. Claims relating to products derived from such 5-HTT polynucleotide or 5-HTT protein (i.e. claim 16) are also unclear in the sense of Article 6 PCT.

A polynucleotide or a protein should be defined in terms of its chemical structure (i.e. its sequence).

In addition, no protein sequences are disclosed in the present application. Moreover, it appears from the description, that the polynucleotide of SEQ ID NO: 1 or 2 corresponds to the 5' non-translated region of the 5-HTT gene. Therefore, no protein sequence can be deduced either from SEQ ID NO: 1 nor SEQ ID NO: 2.

The present application, discloses also various polymorphisms in the 5-HTT gene that are identified with reference to polynucleotide sequences identified by accession numbers. The applicant's attention is brought to the fact that accession numbers are however deemed to be unclear in the sense of Article 6 PCT as the information provided by such accession numbers can change over time.

V.7.4. In view of the fact that the only polymorphism in the 5-HTT gene which is linked to the susceptibility to IBS is located in the 5' non-translated region of the 5-HTT gene and the fact that the other polymorphisms listed in Table 1 are all located in the introns of the 5-HTT gene, there is no support in the sense of Article 6 PCT as to any polymorphism located in the coding region of the 5-HTT gene (i.e. protein) which is linked to the susceptibility to IBS.
Claim 15, insofar as relating to a protein comprising a polymorphism which causes suceptibility to gastrointestinal disease, is therefore not supported by the description as required by Article 6 PCT.

In the absence of any polymorphism located in the coding region of the 5-HTT gene which is linked to the susceptibility to IBS, it appears that by culturing a host cell transformed or transfected with a vector incorporating a 5-HTT polynucleotide comprising a polymorphism that causes susceptibility to gastrointestinal disease located in the non-translated region of said polynucleotide in order to expresse the protein encoded by said polynucleotide, the protein thus expressed **will not contain a polymorphism** causing susceptibility to gastrointestinal disease. Claim 17, which relates to a process for the preparation of a 5-HTT protein that comprises a polymorphism linked to the susceptibility to gastrointestinal diseases lacks therefore support in the meaning of Article 6 PCT and the application does not fulfill the requirements of Article 5 PCT.

Independently of the above objection, the applicant's attention is brougth to the fact that expressing a 5-HTT protein in an expression vector is well known in the art (see document D4 which discloses the expression of the 5-HTT protein in a baculovirus system).

V.7.5. A lack of clarity in the sense of Article 6 PCT arises from the wrong back reference to claim 14 in claim 17. Claim 17 relates to a process which comprises "culturing a host cell transformed or transfected with a vector according to claim 14", however claim 14 relates to a kit and not to a vector. As the only claim which is relating to a vector is claim 16, claim 17 should be read as comprising "culturing a host cell transformed or transfected with a vector according to claim 16".

V.7.6. Lastly, it is clear from the present application that the compounds listed on page 16, line 28 to page 17, line 7 can be used to treat patients diagnosed with IBS, however the present application does not provide any support for a prophylactic action of such compounds. Therefore, claims 10-12 insofar as relating to the use of such compounds in the manufacture of a medicament for use in a prophylactic treatment of a patient susceptible to gastrointestinal diseases appear to lack support in the meaning of Article 6 PCT.